

# Sediment and Dye Concentration Effects on Fluorescence

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## ABSTRACT

**B**ECAUSE of the relatively large sediment concentrations sometimes found on upland regions, adsorption of fluorescent dye onto sediment may be of concern. A laboratory study was conducted to identify the effects of sediment and dye concentration on adsorption. Sediment and dye concentration were both found to significantly affect adsorptive dye loss of rhodamine WT and sulpho rhodamine B.

Regression equations were developed which related dye adsorption to sediment and dye concentration. For a particular soil and dye material, regression equations may be used to correct for adsorptive dye loss. Sulpho rhodamine B is recommended as the dye of choice for the given experimental conditions.

## INTRODUCTION

Tracing techniques have been successfully utilized to characterize the behavior and movement of water. Floats, chemical salts, actual contaminants and radioisotopes have all been used as tracers (Wilson, 1968). Recently, fluorescent dyes have proven to be excellent tracer materials (Chase and Payne, 1968).

Fluorescence results from the instantaneous emission of light from a molecule which has absorbed light. A fluorescent chemical compound will absorb light (exciting light) at one wavelength and emit it at a longer wavelength (fluorescent light). For fluorescent dye in solution, the intensity of the fluorescent light is proportional to the dye concentration. The device used to measure the intensity of fluorescent light is a fluorometer.

The fluorometer provides a primary filter between the light source and sample. The desired wavelength of exciting light is transmitted by the primary filter. A secondary filter, located between the sample and a photodetector, is used to select the wavelength of emitted light. The photodetector produces a current which is proportional to the intensity of the fluorescent light. The current is then amplified to provide a reading on a meter or recorder.

Fluorescent dyes are economical and can be measured quantitatively in concentrations as low as a few parts per trillion. The low tracer concentration requirement makes

handling of the fluorescent material relatively easy, allowing for portable operation of the fluorometer. Direct readings of dye movement over a period of time may be made using a continuous flow sample. Readings for individual samples, which can be collected and carried back to a laboratory for analysis, may also be obtained.

Fluorescent dyes have been used extensively in stream and river studies. Information on fluorometric procedures for discharge determination, velocity measurement and dispersion testing has been presented (Wright and Collings, 1964; Replogle et al., 1966; Chase and Payne, 1968; and Hubbard et al., 1982). Kilpatrick (1968) and Morgan et al. (1977) used dye-dilution techniques for flow calibration. Dye requirements for slug injections into streams were described by Kilpatrick (1970).

A large number of fluorescent dyes are commercially available. However, only a few have been utilized in water investigations. The best tracers are water soluble, easily detectable, harmless in low concentrations, inexpensive and stable (Wilson, 1968).

Fluorescein was one of the first fluorescent dyes used in hydrologic studies. However, fluorescein was highly susceptible to photochemical decay and background interference. The dye of choice during the early 1960's was rhodamine B. Rhodamine B, which was readily adsorbed onto sediment, was soon replaced by sulpho rhodamine B (pontacyl brilliant pink). Less expensive rhodamine WT was later developed and has been used widely in hydrologic studies. An extensive evaluation of several fluorescent dyes used for water tracing was conducted by Smart and Laidlaw (1977), who found that lissamine FF demonstrated a relatively high resistance to adsorptive dye loss.

Although fluorometric procedures have been widely utilized in stream and river studies, limited use of fluorescent dyes has occurred in upland areas. Many existing discharge and velocity measuring procedures are not well suited for use with the small runoff quantities found in overland flow and shallow concentrated flow regions. Therefore, dye tracing techniques may be useful in characterizing hydraulic variables on upland areas.

Chemical dilution techniques may be used to make discharge measurements. A tracer of known concentration may be added to a channel and by knowing the degree of dilution at a downstream sampling point, the channel discharge can be determined (Replogle et al., 1966). Depending upon study site characteristics, the tracer may be added continuously or by slug injection.

The slug injection technique may also be used to determine flow velocity. A slug of dye may be injected into a channel at a sufficient upstream distance to allow

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TABLE 1. PARTICLE SIZE ANALYSIS AND ORGANIC MATTER CONCENTRATION OF THE BENCHMARK SOILS

Soil series	Particle size analysis, %			Organic matter, %
	Sand	Silt	Clay	
Barnes	39.3	37.7	23.0	2.34
Caribou	39.3	48.8	11.9	2.55
Houston Black	12.3	41.9	45.8	1.61

complete mixing. By dividing travel distance by travel time, flow velocity can be determined.

#### DYE ADSORPTION ONTO SEDIMENT

Adsorption of fluorescent dye onto sediment may be of concern on upland regions. Sediment concentration of runoff on these areas may be substantially larger than that typically found in streams and rivers. If dye loss is significant, corrections for discharge measurements must be made. A laboratory study was therefore conducted to determine the effects of sediment content and dye concentration on adsorption for selected soils.

#### Soil Characteristics

Three benchmark soils, Barnes, Caribou and Houston Black, were used to evaluate adsorptive dye loss. Each of these series represent important agricultural soils used principally for cropland. The soils were selected because of their widely varying chemical and physical characteristics. Dye adsorption for these soils should be indicative of losses expected for many cropland sites.

Particle size analyses and organic matter concentration of each of the benchmark soils is shown in Table 1. The experimental procedures outlined by Day (1965) were used for particle size analysis while organic matter concentration was determined using methods developed by Walkley and Black (1934). The percentage

of clay particles ranged from 11.9 to 45.8% for the Caribou and Houston Black soils, respectively. Concentration of organic matter varied from 1.61 to 2.55% for the Houston Black and Caribou soils, respectively.

The Barnes series (fine-loamy, mixed Udic Haploborolls), consists of deep, well-drained soils formed in glacial till on uplands. The surface layer of this soil, obtained in Pierce County, ND, is black loam approximately 18 cm thick. Most of these soils are used for cropland.

Aroostook County, Maine was the location from which the Caribou soil (coarse-loamy, frigid Typic Haplorthods) was obtained. The Caribou series consists of deep, well-drained soils on uplands. These soils formed in glacial till derived mainly from calcareous slate and limestone. Typically, these soils have a dark brown gravelly loam surface approximately 20 cm thick.

The Houston Black series (montmorillonitic, thermic, udic Pellusterts), a sample of which was obtained from Bell County, Texas, consists of deep moderately well drained soils on nearly level to gently sloping areas on uplands. The soils formed in calcareous marine clays and marls. In a representative profile, the surface layer is very dark gray clay about 60 cm thick.

#### Dye Characteristics

The fluorescent dyes used in the laboratory study were lissamine FF, rhodamine WT and sulpho rhodamine B. These tracer materials have previously been found to be stable and relatively resistant to adsorption (Smart and Laidlaw, 1977). Sensitivity, minimum detectable concentration and linear range for the three dyes are shown in Table 2.

Sensitivity is defined as the gradient of the calibration curve for the most sensitive scale. Minimum detectability is a reading 10% in excess of background fluorescence for distilled water or one scale unit, whichever is greater. For the linear calibration range, a change in the

TABLE 2. SENSITIVITY\*, MINIMUM DETECTABILITY\*, LINEAR RANGE AND EXPERIMENTAL DYE CONCENTRATIONS

Dye	Sensitivity,† μg/liter/scale unit	Minimum‡ detectability, μg/L	Linear range,§ μg/L	Experimental dye concentrations, μg/L
Lissamine FF	0.11	0.29	65-800	598
				1,010
				2,630
				5,200
Rhodamine WT	0.013	0.013	0.43-100	0.440
				5.00
				52.0
				538
Sulpho rhodamine B	0.061	0.061	5.0-1000	5,080
				11
				123
				1,040
				9,490
				345,000

\*Data are from Smart and Laidlaw (1977).

†Sensitivity is the gradient of the calibration curve for the most sensitive scale.

‡Minimum detectability is a reading 10% in excess of background fluorescence for distilled water or one scale unit, whichever is greater.

§For the linear calibration range, a change in the instrument reading is linearly related to variation in dye concentration.

instrument reading is linearly related to the variation in dye concentration. Slight differences in each of these values may occur from one fluorometer to another.

Each of these dyes are water soluble, strongly fluorescent and relatively stable. Between pH 4.0 and pH 10.0, no significant change in fluorescence occurs with lissamine FF or sulpho rhodamine B (Smart and Laidlaw, 1977). Fluorescence of rhodamine WT, however, is substantially affected below pH 5.0.

Fluorescence varies inversely with temperature depending on the dye. Photochemical decomposition of fluorescent dyes may also occur. Temperature and photochemical decay coefficients have been reported for each of the dyes used in the laboratory study (Smart and Laidlaw, 1977).

### Experimental Procedures to Measure Dye Adsorption

The soils were air dried and sieved through a 2 mm screen prior to testing. For a given dye concentration, as shown in Table 2, soil was added to a 400 mL solution at rates of 2.5, 10, 40, 70, and 100 g/L. The samples were stirred and allowed to stand for one hour.

Sediment was removed from the solution by filtering. A fluorometer was then used to determine remaining fluorescence of the solution. Throughout the testing procedure, the solution was maintained at a temperature of 25°C.

### Factors Affecting Dye Adsorption

Multiple linear regression analysis of the experimental data was utilized to determine the effects of sediment and dye concentration on adsorption for each of the soils. In the statistical analysis, the following equation was evaluated:

$$Y_{ij} = \mu + \beta_1 (X_{1j} - \bar{X}_1) + \beta_2 (X_{2j} - \bar{X}_2) + \beta_{1i} (X_{1j} - \bar{X}_1) + \beta_{2i} (X_{2j} - \bar{X}_2) + T_i + e_{ij}$$

where:  $Y_{ij}$  = percentage of initial fluorescence for the  $i^{\text{th}}$  soil and  $j^{\text{th}}$  level of sediment and dye concentration;  $\mu$  = sample mean;  $\beta_1$  = regression coefficient for sediment concentration over all soils;  $X_{1j}$  = sediment concentration;  $\bar{X}_1$  = mean sediment concentration;  $\beta_2$  = regression coefficient for dye concentration over all soils;  $X_{2j}$  = dye concentration;  $\bar{X}_2$  = mean dye concentration;  $\beta_{1i}$  = regression coefficient for sediment content for the  $i^{\text{th}}$  soil;  $\beta_{2i}$  = regression coefficient for dye concentration for the  $i^{\text{th}}$  soil;  $T_i$  = additive effect for the  $i^{\text{th}}$  soil; and  $e_{ij}$  = random error term for the unexplained variation.

Multiple linear regression analysis was used to determine the F ratios shown in Table 3. The F ratio can be used to identify the degree of significance of selected variables. For each of the fluorescent dyes, the variable most affecting adsorption was sediment concentration.

Only variations in sediment concentration significantly affected adsorption of lissamine FF. However, sediment and dye concentration were both found to influence adsorptive dye loss of rhodamine WT and sulpho rhodamine B for each of the soils. For these fluorescent dyes, the response due to sediment concentration was also found to vary significantly between soils as indicated in Table 3 by the soil-sediment interaction. Regression equations to correct for dye adsorption should be

TABLE 3. F RATIOS FOR REGRESSION ANALYSIS OF PERCENTAGE OF INITIAL FLUORESCENCE WITH SOIL SERIES, SEDIMENT CONCENTRATION AND DYE CONCENTRATION

Source of variation	F Ratio		
	Lissamine FF	Rhodamine WT	Sulpho rhodamine B
Soil series	1.8	17.5*	170.2*
Sediment concentration	303.2*	204.2*	836.9*
Dye concentration	0.4	22.0*	9.2*
Soil-sediment interaction	0.9	5.6*	83.4*
Soil-dye interaction	1.5	0.6	0.5

\*Statistically significant at  $\alpha = 0.10$  level.

developed for each soil and should include sediment and dye concentration as variables.

### Estimating Dye Adsorption

Once the factors affecting adsorptive dye loss had been identified, equations for estimating dye adsorption were developed. Regression equations were obtained which related adsorption, expressed as a percentage of initial fluorescence, to sediment and dye concentration. By using the equations shown in Table 4, corrections for adsorptive dye loss can be made for each soil.

For the three soils tested, the regression equations should provide accurate estimates of adsorptive dye loss. It can be seen from Table 4 that the coefficient of determination varied substantially between soils. When all three soils are considered, the best statistical fit occurred for sulpho rhodamine B.

### Dye Selection

In selecting a dye for tracer studies, several factors should be considered. The sensitivity, minimum detectability and linear calibration range of the dye should be examined. The amount of dye adsorption onto sediment is also of concern. In addition, the accuracy of correction factors for dye adsorption should be considered.

Adsorptive dye loss was less for lissamine FF than for the other fluorescent materials. However, the minimum detectability was much greater and the linear range much less for lissamine FF than for rhodamine WT or sulpho rhodamine B. Although still acceptable, the minimum detectability for sulpho rhodamine B was approximately five times greater than that displayed by rhodamine WT.

Rhodamine WT and sulpho rhodamine B displayed similar dye adsorptive characteristics. The regression equations describing dye adsorption for sulpho rhodamine B, however, provided the best statistical fit. Thus, sulpho rhodamine B is recommended as the dye of choice for the given experimental conditions.

### DISCUSSION

This laboratory study was conducted to evaluate the effect of sediment and dye concentration on adsorption for selected soils. Only a few of a large number of interrelated variables were evaluated in this investigation. Chemical variables, slope morphology and

TABLE 4. STATISTICAL ANALYSIS OF DYE ADSORPTION EQUATIONS\*

Dye	Soil	Regression coefficient.		Intercept, $\alpha$	Coefficient of determination, $r^2$
		$\beta_1$	$\beta_2$		
Lissamine FF	Barnes	-0.094	173	98.4	0.916
	Caribou	-0.093	188	98.5	0.905
	Houston Black	-0.079	217	96.9	0.740
Rhodamine WT	Barnes	-0.281	1600	95.6	0.806
	Caribou	-0.155	953	97.0	0.759
	Houston Black	-0.253	1620	92.4	0.767
Sulpho rhodamine B	Barnes	-0.383	5.74	96.0	0.968
	Caribou	-0.103	12.0	98.1	0.766
	Houston Black	-0.324	6.90	96.8	0.924

\*The regression coefficients and intercept were determined for the equation:

Percentage of initial fluorescence =  $\beta_1$  (sediment concentration) +  $\beta_2$  (dye concentration) +  $\alpha$   
where sediment and dye concentration are given in g/L.

soil factors may also affect dye dilution measurements. These factors should be considered when fluorometric procedures are used under actual field conditions.

Temperature, pH, salinity and chlorine concentration may affect flow measurements. Natural fluorescence, photochemical and chemical decay may be of concern. Dye toxicity and the cost of tracer materials are other important considerations.

When making discharge measurements in the field, attention should be given to slope length, gradient, and shape. Soil surface roughness and the existence of localized irregularities along the flow path may influence flow measurements. The density and distribution of surface residue and coarse soil material should be considered. Adsorption of dye onto soil material as the flow moves over the soil surface may also occur.

Soil infiltration rate may affect flow measurements. Infiltration rate is dependent upon particle size distribution, particle shape, porosity, soil density, soil structure and antecedent water conditions. Soil organic matter and clay mineralogy may also influence dye adsorption.

Testing of fluorometric procedures for upland flow characterization under field conditions is needed. Adsorption of dye for selected types and rates of crop residue should be evaluated. The interrelationship of the variables affecting dye dilution measurements should also be examined.

## SUMMARY AND CONCLUSIONS

Although fluorescent dyes have been widely utilized in stream and river studies, they have received only limited use for upland flow characterization. Many existing discharge and velocity measuring procedures are not well suited for use with the small runoff quantities found in overland flow and shallow concentrated flow regions. Therefore, dye tracing techniques may be useful in measuring hydraulic variables on upland areas.

Adsorption of fluorescent dye onto sediment may be of concern on upland regions. A laboratory study was conducted to determine the effects of adsorption due to sediment and dye concentration for selected soils. Sediment and dye concentration were found to significantly affect dye loss of rhodamine WT and sulpho rhodamine B. For a particular soil and dye material, regression equations can be developed to estimate adsorptive dye loss.

When selecting a fluorescent dye for flow characterization, sensitivity, minimum detectability and linear calibration range of the dye should be considered. The amount of dye adsorption onto sediment and the accuracy of equations to correct for adsorption should also be of concern. When these factors were taken into consideration, the dye of choice for the given experimental conditions was sulpho rhodamine B.

Use of fluorescent dyes for upland flow characterization has considerable potential. If discharge measurements are made on upland areas, use of correction factors for estimating dye adsorption may be necessary. Dye tracing techniques could be utilized to greatly expand information on hydraulic parameters on upland areas. Testing of dye dilution procedures under field conditions is recommended.

## References

1. Chase, E. B. and F. N. Payne. 1968. Selected techniques in water resources investigations. U. S. Geological Survey Water-Supply Paper 1892, 43 p.
2. Day, P. R. 1965. Particle fractionation and particle size analysis. In: C. A. Black (ed) Methods of Soil Analysis, Part 1. Amer. Soc. Agron., Madison, WI., p. 545-567.
3. Hubbard, E. F., F. A. Kilpatrick, L. A. Martens and J. F. Wilson. 1982. Measurement of time of travel and dispersion in streams by dye tracing. Techniques of water-resources investigations of the U.S. Geological Survey, Book 3 (Applications of Hydraulics), Chapter A9, 44 p.
4. Kilpatrick, F. A. 1968. Flow calibration by dye-dilution measurement. Civ. Eng. 38(2): 74-76.
5. Kilpatrick, F. A. 1970. Dosage requirements for slug injections of rhodamine BA and WT dyes. U. S. Geological Survey Professional Paper 700-B, p. 250-253.
6. Morgan, W. H., D. Kempf and R. E. Phillips. 1977. Validation of use of dye-dilution method for flow measurement in large open and closed channel flows. National Bureau of Standards Special Publication 484, p. 366-394.
7. Replogle, J. A., L. E. Meyers and K. J. Brust. 1966. Flow measurements with fluorescent tracers. J. Hydraulics Div., ASCE, No. HYS-1-14.
8. Smart, P. L. and I. M. S. Laidlaw. 1977. An evaluation of some fluorescent dyes for water tracing. Water Resources Res. 13(1):15-33.
9. Walkley, A. and I. A. Black. 1934. An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chronic acid titration method. Soil Sci. 37:29-38.
10. Wilson, J. F. 1968. Fluorometric procedures for dye tracing. Techniques of water-resources investigations of the U.S. Geological Survey, Book 3 (Applications of Hydraulics), Chapter A12, 31 p.
11. Wright, R. R. and M. R. Collings. 1964. Application of fluorescent tracing techniques to hydrologic studies. J. Amer. Water Works Assoc. 56(6):748-754.